ɅERICAN

.L·OF BOTANY

TABLE

ᴧL PUBLICATION OF THE

ᴄAL. SOCIETY OF AMERICA

VOLUME VII 1920

TH THIRTY-FIVE PLATES AND ONE HUNDRED AND SEVENTY TEXT FIGURES

PUBLISHED
IN COOPERATION WITH THE BOTANICAL SOCIETY OF AMERICA
BY THE
BROOKLYN BOTANIC GARDEN
AT 41 NORTH QUEEN STREET, LANCASTER, PA.

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ERRATA, VOLUME VII

Page 139, line two in legend, read Fig. 67 for Fig. 87.
Page 148, line 29, read plant for plane.

AMERICAN JOURNAL OF BOTANY

VOL. VII JANUARY, 1920 No. 1

THE INJECTION OF CHEMICALS INTO CHESTNUT TREES[1]

CAROLINE RUMBOLD

The rapid spread of the chestnut bark disease caused by *Endothia parasitica* (Murr.) A. and A. in the eastern part of the United States during the past ten years and the resultant appeals for help from owners of ornamental chestnut trees and of chestnut orchards, reluctant to lose their trees, were the reasons for this experimentation.

As a rule the fungous diseases of plants are such that the application of sprays, crop rotation, fertilizers, and sanitary methods of cultivation prevent or hold them in check. This disease, however, like many others to which trees especially are subject, can not be treated in this way. The cause of the sickness is in a part which can not be reached by any outside application of medicine, fertilization of the soil will not help, nor will sanitation prevent, at least in many parts of the eastern states.

The customary method of keeping such a disease in check has been to cut away and burn the diseased parts or the entire trees. The money value of the individual trees caused discontent with this method.

Experiments on tree injection were undertaken as a possible remedy. It was believed that from its nature this treatment could not be applied to forest trees. Only such trees as had a definite commercial or aesthetic individual value would repay the requisite cost and trouble.

HISTORY OF PLANT INJECTIONS

The idea of introducing foreign substances into plants is two centuries old. In 1709 Magnol (cited by Sachs, 1) introduced colored solutions into plants in order to find through what channels the sap passed. These first injections were made by placing the cut stems of leaves, twigs, or flowers in the solutions. McNab (2) was the first to put lithium into trees. He used caesium as well. About this time Pfitzer (3) injected salts of thal-

[1] The Pennsylvania Chestnut Tree Blight Commission was responsible for the starting of this experimental work in 1912; Investigations in Forest Pathology, Bureau of Plant Industry, for its continuation in 1913–1914 and in part for its continuation until 1918. The University of Pennsylvania furnished laboratory facilities, greenhouse, and many supplies. To Mr. Harold Peirce of Philadelphia, Secretary of the Commission, belongs the credit that these experiments were continued to their present stage.

lium. In 1887, Gaunersdorfer (4) published the results of seven years of experimentation on the effect of lithium sulphate on plants. He injected small conifers without injuring them and found that the plants finally eliminated the salt introduced into them through the roots by throwing it off with the leaves and bark. He believed young shoots, leaves, and reproductive organs were protected from the lithium by the lack of lignified water-transferring tissue. Physiologists such as Sachs (5), Strasburger (6), Wieler (7), and Pfeffer (8) established the fact that some substances foreign to plant tissues could be safely conducted through them. At the same time a large number of substances were found to be poisonous. In general, the response given by the plants to the poisons resembled that given by animals, *i.e.*, a very small amount of poison could be introduced into them without injury or noticeable change; a still larger amount increased their activities, often their growth; a larger amount retarded their activities, while still more killed. A plant could furthermore become accustomed to a poison to a certain limit, provided the poison was introduced into it in small quantities at first and these doses gradually were increased. Doses could in the end be administered without detriment that would otherwise have killed at once.

The idea of injecting trees for purposes of wood preservation is also old. In 1840 and 1841, Boucherie (9) published accounts of experiments in which chemicals were injected into living trees. His method of injection killed the tree. The introduced liquid was distributed up and down the trunk, the injected area decreasing rapidly in breadth toward the roots. Fall was the best season for a complete saturation by this method, but it could be done in the spring. Coniferous trees were an exception because sap movement took place in them throughout the winter. Different substances were absorbed at different rates; neutral salts penetrated the wood in large quantities, acids and alkalies to a less extent. If there were hard knots or rotten spots at the base of the tree, the whole strip of wood above them would not be saturated at all. The same was true of the old wood of hard wood plants. Boucherie's ideas were used by Shevyrev in his work.

The first paper on tree injection for purposes of medication was that by Ivan Shezyrez (J. Shevyrev, Schewirew or Chewyreuv) (10). The most of Shevyrev's experiments on the injection of living trees were made with stains for the purpose of establishing the fact that solutions of substances foreign to tree tissues could safely be introduced into trees. He mentions injecting grape vines with copper sulphate but does not give the results. He describes his methods of injection and his theories as to tree injection as follows:

The best time for injection is the late summer and fall. The liquid is distributed to all parts of the tree, with the exception of the dead portions. The liquid enters the roots as well as the leaves, twigs, and fruits. This current takes the place of the sap, ascending and descending, the only dif-

ference being that it is an unusual (extraradicate) instead of the usual (radicate) current. The duration of this created second sap movement does not exceed five days. The most intensive absorption takes place at the beginning, gradually diminishes, and ceases entirely in from three to five days. He believed this diminution and cessation due to the obstruction of the vessels. Shevyrev found that the weather greatly influenced the rate of intake; he made a record of the hourly intake of an injected grape vine and of the weather for a period of three days, which showed that the consumption at night was less in quantity than that in the day, regardless of the weather.

Shevyrev's experiments were made primarily for the purpose of destroying such insects as injure plants by burrowing beneath the bark. He believed, however, that fungous diseases could be cured by the same method.

Shevyrev did not continue his experiments. The last paper (11) he published on the subject describes and criticizes the injection experiments of some Russian workers who had been treating diseased trees. He speaks of the experiments of K. K. Reshkv or K. Reschko in the Crimea, to which no other reference could be found by the present writer. Reschko treated in 1901, according to Shevyrev, a thousand trees suffering from chlorosis by introducing iron sulphate into canals cut in the bases of the diseased trees. The distribution of the substance was found to be irregular, so that individual branches were found to be uninjected.

Pachassky (12), in a governmental report of 1903, reported favorably on the injection of iron sulphate either in powder or in solution in the treatment of diseased fruit trees.

C. A. Mokrjetsky (S. A. Mokrzecki or Mokrzhetski) (13), in governmental reports of 1902 and 1903, tells of injecting more than 500 trees, the method of injection being analogous to Shevyrev's. Diseased apple trees were cured with iron sulphate, gummosis of apple, pear, and other trees with 1 percent salicylic acid. He injected "nutrient solutions" into frost bitten trees, which recovered rapidly after treatment and grew three times as much as the untreated trees. Another article (14) "Über die innere Therapie der Pflanzen" explains his work in more detail. The two methods of injection used are explained. One of them consists of inserting the dry salt in holes bored in the tree trunk. These holes are then closed with grafting wax. In the other method solutions are injected. The hole made in the trunk for the purpose of injecting is bored with a brace and bit which passes through a metal tube embedded in the tree. A side outlet in this tube is connected by a rubber tube with a jar containing the solution to be injected. As the hole is bored by the brace and bit the solution passes into it, thus shutting out the air from the wound. Diseased trees were injected with copper sulphate, calcium cyanide, and arsenic in 1/100 percent concentration, with inconclusive results. Iron sulphate in 0.05–0.25 percent solutions (amount injected not stated), or the dry salt, 12

grams for trees with 16–25 cm. diameter, cured apple trees of disease and insects. Mokrjetsky stated that he was carrying on more experiments as he believed that the fertilization of plants with such injected salts often cured them at the same time of diseases.

The best reports on tree injection so far printed are the Russian. Most of the experiments were made in the Crimea. Here many of the fruit trees appear to suffer from malnutrition, according to Mokrjetsky (14), and the iron sulphate appeared to act as a most efficient fertilizer. The dry, hot summer climate of this region favored the rapid consumption and transfer of the injected solutions, to which the trees reacted in a striking manner. No reports have been found as to the length of time the injected iron sulphate acts as a fertilizer, except a statement by Mokrjetsky that in the spring following the injection the buds on the fruit trees were numerous and large. The Russian experimenters appear to have stopped, unfortunately, before they had concluded their work. In 1912 the writer received a letter from Shevyrev saying that he was unable to continue the injections and hoped that the work would be carried on in this country.

A series of short papers by German, French, and American workers followed Shevyrev's publications.

Roth (15) in 1896 described a method and apparatus for injecting trees.

Mangin (16) in 1898 unfavorably criticized plant injection, especially the idea that grape vines could be protected from fungi by the injection of salts. He regarded plant injection impracticable in agriculture.

Goff (17) found the injection of water into the roots of newly transplanted trees to be beneficial. He described his apparatus and method of injection. His experiments showed that this treatment hastened the initial growth of the trees.

Bolley (18) in three reports described experiments in stimulating tree growth by injecting liquid solutions into the trunk. He successfully treated diseased apple and plum trees with a formaldehyde solution of 1/2 to 2 parts per 1000 of water. He reported that the effect of injected solutions on parasitic diseases was inconclusive.

Simon (19) reported that he successfully injected apple and peach trees, grape vines, and potatoes. Water solutions of purin and potassium nitrate and nutrient solutions were used. Copper sulphate injected into grape vines was at first injurious, but later the vines produced new leaves free from fungi.

Fron (20), using Simon's method of injection, treated pear trees with solutions of iron sulphate and calcium nitrate. The vigor of the trees appeared to be increased, but the improvement was confined to portions of the trees only. He believed this method of little practical value in fruit culture.

Coffigniez (21) experimented about the same time with iron sulphate and fruit trees in the control of fungus diseases.

Sanford (22) published a note on the effect of potassic cyanide on the scale. He considered the insertion of the salt beneficial to the tree. This result was disputed by Surface (23), Shattuck (24), Moore and Ruggles (25), and Flint (26). The experiments of the latter-named workers showed the injurious effects of a concentrated solution of cyanide of potassium on plant tissues. No attempt was made to try the effect of a gradual impregnation with dilute solutions of the salt. These articles are reviewed by Elliott (27) in a publication which describes the effect of cyanide of potassium on woody and herbaceous plants. Elliott worked with a killing solution, as he inserted the crystals under the bark and epidermis of the plants and depended on the sap to dissolve the crystals. The reactions of the plants were extreme, the tissues in the path of the solution being killed when the solution was concentrated. He found that the weather had a decided effect on the kind of reaction and the time of response of the tree. Trees treated on cool, damp days responded more slowly and showed less extensive injury than those treated on hot, dry days. He found also that the rate of transpiration affected to some extent the path of the solution. When transpiration was slow the solution passed into the cells surrounding the vessels; when it was rapid the solution appeared to pass through the vessels without going into the surrounding cells.

Rankin (28) injected ten chestnut trees with lithium nitrate solutions varying from 0.1 to 0.002 percent. His analysis of the trees showed that the salt had penetrated the bark and sapwood above and below the place of injection. When trees were less than three inches in diameter there was complete penetration of the heartwood, but in trees of greater diameter the penetration did not seem to follow a definite rule, the heartwood sometimes being impregnated, sometimes not. The tip of the trees was found impregnated. Aside from blotching of leaves the trees were not injured.

The Russian and American papers give the most definite reports, both as to practical methods of injecting and as to the results of the injection.

The Problem

In studying such a problem as the injection of a tree, a number of fundamental considerations present themselves:

A substance in solution injected into a tree generally passes through those vessels in the neighborhood of the place of injection through which the crude sap ascends from the roots to the leaves. It can also descend through those vessels, but in all of this there is lacking that persistent passing and returning of a stream such as constantly bathes the cells of the animal body.

The streams passing through this region, besides varying constantly in rate of flow, content, concentration, and acidity, are also under different atmospheric pressures.

The physical attributes of the cells must be considered. The surface of the cell walls, aside from the semipermeable membranes of the living cells in the region of the vessels, offer surface films which are constantly within the field of absorptive and adsorptive forces.

The chemical content of the sap may be changed by the injections, insoluble mineral compounds may be formed and toxins made harmless thereby.

These conditions at this stage of experimentation called for a great deal of empirical experimental work with chestnut trees.

In order to study this subject fundamentally, an attempt was made to answer by means of experimentation the following questions: (1) What substances can be injected into living chestnut trees? (2) When can they be injected? (3) Where does the injected material go? (4) What is the effect on the chestnut tree? (5) What is the effect on the fungus growing parasitically on the trees?

The present record gives the results of five years' experimental work. The work here reported is not complete. The propositions offered for solution have, however, been so varied in character that it seemed proper to bring together in this and a succeeding paper the different results so far secured, since this work must for the present be laid aside.

Experimental Procedure

Experimental Plots

The principal experimental plots of trees were in the center of a blight-infected chestnut orchard of some three hundred-odd acres' extent, located in southeastern Pennsylvania. They were on top of a hill about 500 feet above sea level. This region is hilly and originally was covered by a mixed forest of conifers and deciduous trees, a large proportion of the deciduous trees being chestnuts. The fact that this is the fourth generation of chestnut trees growing here since the Revolutionary War shows how favorable is this region to the growth of chestnut.[2]

Trees

The trees used in the experiments were orchard trees, for the most part Paragon scions grafted on native chestnut stock, *Castanea dentata*. The trees in the plots varied in age according to the year of grafting. One set was about ten, the other fourteen years old. They were short, stocky trees

[2] An analysis for alkali content was made of the soil by the Bureau of Soils, Department of Agriculture.

K_2O	trace	
CaO	0.27%	No CO_2 from carbonates.
MgO	0.68%	
P_2O_5	trace	
N	0.08%	
Li	none	

in form, the greatest height being about five meters, the mean height four meters. The orchard had never been pruned or cared for other than by cutting out the underbrush just before the chestnut harvest each fall.

In 1912, when the plots were chosen, they were cleared of underbrush and dead infected trees and were kept clear. Such cankers as threatened soon to girdle the trees were cut out under sanitary conditions. The remaining cankers on the trees were outlined with paint in order to note their rate of growth. The apparatus used in making the injections has been described elsewhere (29)[3].

Injections

Generally two injections were first made in a tree, on opposite sides of the trunk. The next two injections were at right angles to the first two, a little higher up the tree. If more injections followed they were made still higher up in the spaces between the first injections, or on the branches. Observations on the trees injected with substances which blotched the leaves showed that in this way all the branches on the tree could be reached. The hole cut for injection was one centimeter in diameter, and the width of two annual rings of wood into the tree's interior. All the records are based on the intake through holes of this size.

All the substances injected were dissolved in water. This water came from a spring in the orchard and was very lightly mineralized.[4]

[3] In 1915 a different method of injecting trees was tried. In place of the clamps used in the old method, link chains tightened by turnbuckles hold the perforated rubber corks against the tree trunk. The corks are protected from the metal chain by iron washers. Glass T-tubes thrust through the corks introduce the salt solution into the injection holes. The tubes leading from the reservoirs are attached to the vertical ends of the T-tubes. The free ends of the horizontal arms of the tubes are tipped by pieces of rubber tubing. A tempered steel tube shaped like a laboratory cork borer makes the holes in the trunk. It can be driven into the tree through the horizontal arm of the T-tube after the apparatus is in place and the solution fills the T-tube. The solution is cut off by a pinch cock placed over the end of the rubber tip after the drill has been removed. Glass T-tubes were found to be safest for this work because the presence of air bubbles, or leakage in the connections, could be detected easily. It is necessary, for a good injection by this method, that no air enter the injection hole. Seven injections at a time have been made by this method.

[4] Analysis of water by Bureau of Chemistry, Department of Agriculture:

	Mg. per liter
Silica (SiO_2)	5.8
Sulphuric acid (SO_4)	0.8
Bicarbonic acid (H_2CO_3)	10.4
Nitric acid (NO_3)	0.5
Chlorine (Cl)	1.5
Iron (Fe)	0.2
Aluminum (Al)	0.0
Calcium (Ca)	1.2
Magnesium (Mg)	0.9
Potassium (K)	0.7
Sodium (Na)	2.0

The water was tested for heavy metals, lead, copper, etc., none being found.

Measurement of Intake

The intake of an injected tree was measured by weighing the jars containing the solutions. This was done with a small brass beam-balance which recorded the weight in grams. It was assumed that a cubic centimeter of the solution weighed a gram. It was thought that the amount of error caused by this assumption was so small as not to need to be calculated when estimating the amount of substance injected into a tree. Experiment showed that the amount of evaporation from the jars through the parchment covering was so small that it could be ignored. This amount was found to average 40 cc. per month. If the paper cap was torn the average evaporation was 70 cc. per month.

There was also evaporation of the more volatile substances in dilute solution. This could be noticed in the case of the cresols and phenols and of some of the ammonium solutions. The amount of this loss was not tested. The jars containing such solutions had their contents renewed frequently, and an attempt was made by reinjecting to keep the solutions going into the trees rapidly. These precautions were thought to be sufficient to make it unnecessary to calculate either the loss or the concentration of substance due to such evaporation in the experimental work so far attempted.

Substances Injected

The following substances weie injected into the trees:

Inorganic Substances	*Organic Substances*
Copper sulphate	Methyl alcohol
Copper chloride	Formalin
Zinc carbonate	Acetic acid
Mercuric chloride	Formic acid
Potassium chromate	Lactic acid
Potassium bichromate	Citric acid
Barium chloride	Aniline sulphate
Colloidal cuprous hydroxide[5]	Phenol
Colloidal metallic silver	Sodium carbolate
Colloidal metallic mercury	Phenol Sodique[6]
Potassium carbonate	Para nitro phenol
Potassium hydroxide	Ortho nitro phenol
Potassium sulphate	Picric acid
Ammonium carbonate	Meta cresol
Ammonium chloride	Para cresol
Ammonium hydroxide	Thymol
Ammonium sulphate	Pyrocatechin
Sodium carbonate	Pyrogallic acid

[5] I am indebted to H. K. Mulford Company of Philadelphia for these colloidal preparations. The metals were protected in each case by a second colloid.

[6] A patent medicine made of carbolic acid and caustic soda.

Sodium chloride
Sodium hydroxide
Lithium carbonate
Lithium chloride
Lithium sulphate
Lithium hydroxide
Lithium nitrate

Water

Phloroglucin
Oil of bitter almonds
Benzoic acid
Salicylic acid
Bark extracts
Water extract of chestnut tree bark
Water extract of chestnut blight canker

Stains:

Methyl green
Methylene blue
Eosin
Congo red
Trypan blue

All these substances went into the trees in measurable quantities.

Solutions

The solutions were made *gram molecular* except in the case of stains, the bark extracts, formalin, Phenol Sodique, and ammonium hydroxide.

For instance, if a solution of anhydrous sodium carbonate 1/200 G. M. is used, the molecular weight of sodium carbonate is found, which is 106.10. 106.10 grams of salt added to a liter of water makes a gram molecular solution, and a solution 1/200 G. M. means that 1 cc. of the G. M. solution is added to 199 cc. of water.

The chemicals used were bought as chemically pure.

But one substance was injected into a single tree. In a few cases, all of which are indicated in a following list, stronger solutions were used in the later than in the earlier injections in a tree.

Number of Trees Injected

Usually three or more trees were injected with the same substance. The exceptional cases in which fewer than three trees were injected are as follows: But one tree injected: methyl alcohol, Phenol Sodique, oil of bitter almonds, and para cresol. But two trees injected: zinc chloride, barium chloride, colloidal metallic silver, and colloidal metallic mercury. The largest number of trees injected with one salt was thirteen, injected with lithium carbonate solutions of different dilutions. Nineteen check trees were injected with water.

Some of these trees were injected two years in succession, some three years, the greatest number but one year.

The injections were made in 1912, 1913, and 1914. In 1913 a record of the weather was kept together with a record of the daily intake of the trees, so that all remarks on the rate of intake of the trees will be confined to the

records of this year. The records of the previous and succeeding years confirm the 1913 figures.

Rate of Absorption of Injected Substances

This compilation was made from the records of the injections made in 156 Paragon chestnut trees during the growing season of 1913 and of the weather during that period.

The therapeutic bias of the work decidedly limited the scope of the experimental injections, and in consequence data are wanting for a complete record of the rates of intake. The effort was to find the dilution at which a substance entered a tree readily without killing it. When a tree showed injurious effects of an injection, the injection stopped whether it had been going for two days or for a week.

It was the policy in 1913 to inject large quantities of dilute solutions, on the supposition that the dilution decreased the toxicity of the substance near the point of injection. At the same time the tendency of the lignified cell walls to retain the substance was relied on to dilute the solution still further in its passage toward the leaves, so that the latter would not accumulate so much before autumn as to cause them to die. In consequence of this effort the data on the rate of absorption are very incomplete.

Table 1. *Substances, with Their Dilution, Injected Into Trees*

No. of trees	Substance	No. of trees	Substance
	Ammonium:		
4	$(NH)_2CO_3$ 1/100 G.M.	3	Acetic acid
4	$(NH_4)OH$ 1/100 approximately		(2) 1/1000 G.M.
6	$(NH^4)_2SO_4$		(1) 1/3000
	(1) 1/100, changed later to 1/50	3	Benzoic acid
	(2) 1/100		(1) 1/1000
	(2) 1/200		(2) 1/5000
	(1) 1/500	3	Citric acid
2	NH_4Cl 1/200		(1) 1/500
	Sodium:		(1) 1/3000
7	Na_2CO_3		(1) 1/5000
	(4) 1/100	3	Formic acid
	(2) 1/200		(1) 1/1000
	(1) 1/500		(2) 1/6000
4	NaCl	3	Lactic acid
	(3) 1/100, changed to 1/50		(2) 1/1000
	(1) 1/200, changed to 1/50		(1) 1/2000
7	NaOH 1/100 G.M.	4	Picric acid
	Lithium:		(1) 1/500 G.M.
5	LiOH		(1) 1/1000
	(1) 1/200		(2) 1/10000
	(3) 1/500	2	Pyrogallic acid
	(1) 1/1000		(1) 1/100
5	Li_2C		(1) 1/1000
	(1) 1/200	3	Salicylic acid

(1) 1/500, changed to 1/100
(1) 1/5000, changed to 1/1000
(1) 1/1000, changed to 1/500
(1) 1/100

.......LiCl
(2) 1/100
(2) 1/200

Potassium:

.......KOH
(2) 1/100
(2) 1/200

.......K_2CO_3 1/100
.......K_2SO_4 1/100
.......Colloidal copper
(5) 1/3300

.......Colloidal metallic silver
(2) 1/6400

.......Colloidal metallic mercury
(2) 1/6400

.......Potassium bichromate
(1) 1/1000
(2) 1/5000

.......Potassium chromate
(1) 1/5000
(1) 1/1000
(3) 1/1000

.......Copper sulphate
(1) 1/100

(2) 1/5000
(1) 1/10000

3........Aniline sulphate
(3) 1/1000

3........Meta cresol
(3) 1/1000

1........Para cresol
(1) 1/1000

4........Ortho nitro phenol
(2) 1/1000
(2) 1/100000

3........Para nitro phenol
(2) 1/500
(1) 1/1000

1........Oil of bitter almonds
(1) 1/1000

1........Phenol Sodique
(1) 1 cc. to 1,000 cc. H_2O

3........Phloroglucin
(3) 1/1000

3........Pyrocatechin
(1) 1/500
(2) 1/1000

3........Sodium carbolate
(3) 1/1000

3........Thymol
(1) 1/1000
(2) 1/3000

1........Methyl alcohol
(1) 1/100

2........Methylene blue
(2) 1 gm. to 4,000 cc. H_2O

2........Trypan blue
1 gm. to 4,000 cc.

Bark extracts:[7]

3........Healthy bark
(3) 1 cc. to 99 cc. H_2O

2........Canker extract
(2) 10 cc. to 990 H_2O

3........Canker extract-citric acid
(3) Canker ext. 1 cc. to 100 cc. H_2O, with citric acid 1/500 G.M.

13........Water checks

The records of absorption were divided into months for convenience in tabulating. It was found that injections could be made in February and March, when the rate of intake was very slow. The regular injections began in April, but the records for this month were not typical because it

[7] The bark extracts were made by soaking for 24 hours the shredded bark and young wood in spring water. The proportions were 10 cc. of water to 1 g. of healthy bark or of canker tissue. The extracts were filtered before using.

was not until the latter part of the month that injection and weather-recording apparatus were in running order.

The daily intake of the trees was measured each morning, and usually the injections were made in the morning. The hourly intake was not measured, but experience confirmed Shevyrev's observations that the intake by day was greater than by night.

The records of series of injections in individual trees showed that the number of the injection did not influence the amount of solution which went into the tree, i.e., at the sixth injection more cubic centimeters might go into the tree than at the first, or the third injection in the month might be more successful than the first or second. As has been explained, (page 7), care was taken that the new injection was not made directly above or below the old injection hole.

The intake of the trees in the different months was computed and plotted on ruled paper in order that estimates of the rates could be made.

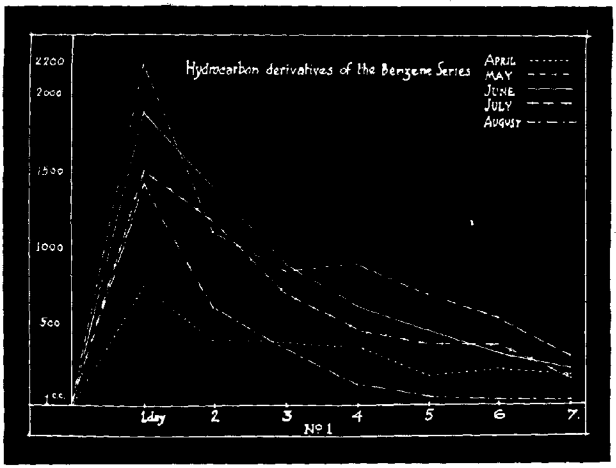

Fig. 1. Graph showing the rate of intake of trees injected with the hydrocarbon derivatives of the benzene series during the spring and summer months.

In computing, the *mean* of the intake of all the injections of a tree during the month represented the monthly intake of that tree per injection.

Plotted curves showing the rate of intake are more varied for April, May, and June than were those for the summer and autumn months. Figures 1, 2, and 3 show the *mean* intake a day per tree reckoned from the day of injection for seven days, of all the trees injected with alkali metals,

organic compounds, and water. As the number of trees being injected varied from month to month, these curves simply approximate the rate of intake.

93 trees are represented in the curves of the hydrocarbon derivatives of

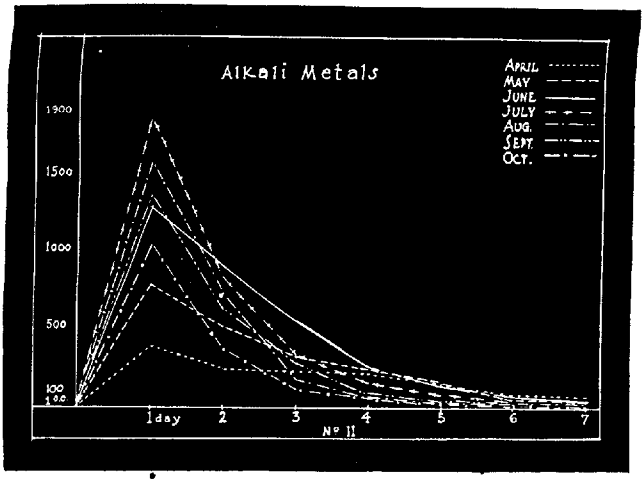

FIG. 2. Graph showing monthly rate of intake of trees injected with the alkali metals.

the benzene series: 7 trees in April, 17 trees in May, 26 trees in June, 33 trees in July, and 10 trees in August. The curve for May, for instance, represents more than 17 injections, for, as has been explained (p. 7), two injections were made in a tree on opposite sides of the trunk, and the daily amount of intake of an injected tree was the mean intake through two injection holes. In May it happened that many of the injections continued for two and three weeks. (The more readily the solution flowed into the tree the fewer were the reinjections.) A number of the trees were injected during one month only, very few for three months, so that no comparison between the intake of a solution by a single tree in the different months could be made. For these reasons the curves approximate the rate of intake, as has been previously stated.

The curves representing the alkali metals are better representations because more trees (121) are represented: 9 in April, 8 in May, 9 in June, 26 in July, 33 in August, 30 in September, and 17 in October. Not only are more trees represented, but more injections to a tree. In spite of the large number of trees, the curves for April, May and June are not typical,

being depressed by the ammonium solutions (counted with the alkali metals), which were injected at this time when comparatively few trees were being treated.

With hardly an exception the rate of intake for the solutions, irrespective of whether they were acid, neutral, or alkaline in reaction, was greater than for water. The exceptions were weak solutions of the ammonium compounds, formic acid 1/6000 G. M., chestnut bark extract, canker extract, and possibly the colloidal solutions of metals.

The typical curve of intake reached its highest point the first 24 hours after injection, then decreased steadily.

Figure 4 shows the rate of intake of an equal number of trees injected July with acids, alkalies, and water. The alkalies surpassed the acids

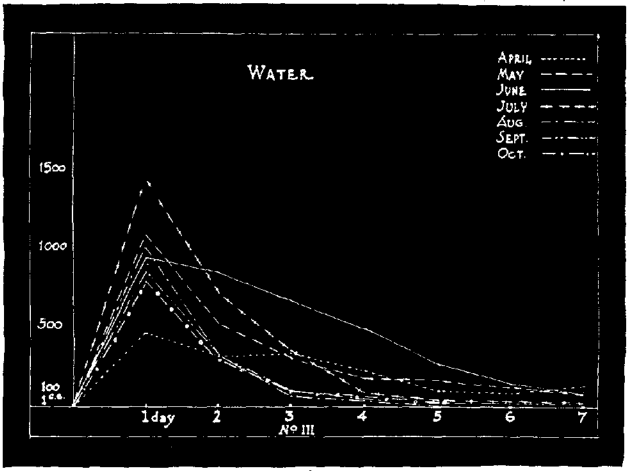

FIG. 3. Graph showing monthly rate of intake of trees injected with water.

in the first 24 hours, but in the second they dropped one half in quantity, and continued to decrease more rapidly than the acids. Because of this rapid decrease in the daily intake of the alkali metals, the trees treated with these compounds usually were injected once a week.

Rankin (28) obtained somewhat similar results when injecting chestnut trees with solutions of lithium nitrate, *i.e.*, the greatest intake was during the first two days and had practically ceased after the fifth and sixth days.

The injections of carbon compounds often ran for three weeks without

a reinjection, sometimes longer. The most marked example of this readiness of intake was a tree injected with para nitro phenol (1/1000 G. M.). This solution flowed into the tree steadily for 41 days without a reinjection. In this time 32½ liters went into the tree through two holes each one centimeter in diameter.

The rate of absorption of the solutions of organic compounds was much

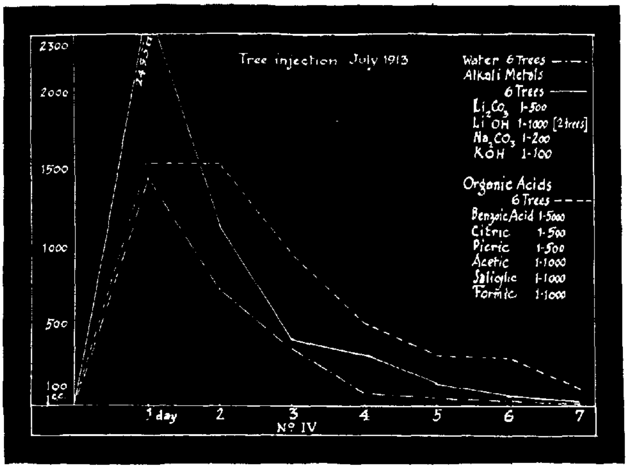

FIG. 4. Graph comparing rate of intake of trees injected during July with water, alkali metals, and organic acids.

greater than the rates of absorption of the solutions of the alkali metals, the heavy metals, and water.

The daily intake of the carbon compounds was extremely irregular. Sometimes the curves seem to indicate that for a short period the intake measured variation in the transpiration of the trees.

The curves of intake of a single tree injected with LiOH 1/200, and those of 3 trees with LiOH 1/500, represented in figures 5 and 6, show how regular was the daily intake of the trees injected with alkali metals. These diagrams also illustrate the fact, common to all the chemicals injected in these experiments, that the greater the concentration of the solutions the greater the intake.

The colloidal solutions of metals were injected into small trees in April before the leaves appeared. All the solutions went in slowly but steadily.

The healthy bark extract went into the trees more readily than the

canker extract. An addition of citric acid to the canker extract increased the intake. These extracts were injected in April and May.

The rate of absorption of solutions of the heavy metals approximated that of solutions of the alkali metals. A solution so concentrated as to be deadly entered the trees more readily than did the more dilute solutions.

During the treatment of the trees a daily record of the weather was kept by means of standard instruments. Some of the weather recording apparatus was not set up until the latter part of April. But after April the

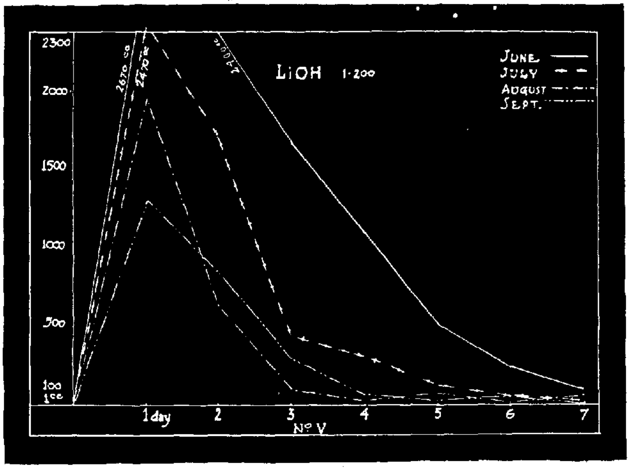

FIG. 5. Graph showing rate of intake of trees injected during the summer months with lithium hydroxide 1/200 G.M.

records were kept until work stopped the last of October. A detailed account of the evaporation and rainfall for this season is given elsewhere (30).

In 1913 the growing season of the chestnut began on April 28, when the leaf buds opened. In May the leaves were nearly mature in size, and flower tassels appeared. By June the leaves were full grown, the flowers had blossomed, and the fruit had set. In July the burs on the trees were half-grown, in August full-grown. In September the nuts began dropping. In October nuts, burs, and leaves dropped from the trees.

Figure 7 shows a monthly compilation of the weather records and of the amount of solution absorbed by a tree per day during each month, every tree injected during the season being used in the computation. The figures in the monthly weather records represented the *mean* of the daily

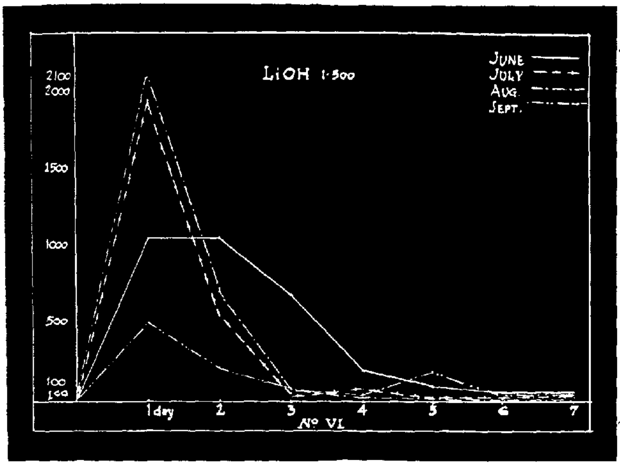

FIG. 6. Graph showing rate of intake of trees injected during the summer months with lithium hydroxide 1/500 G.M.

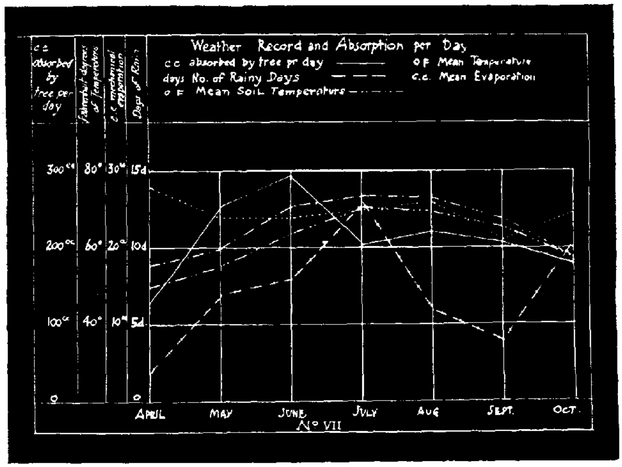

FIG. 7. Graph showing the monthly compilation of the weather records and the amount of solutions absorbed by the trees injected during the year 1913.

records for that month. The amount of rainfall[8] is not recorded in the diagram, because it was noticed that the number of inches of rain which fell on the hill was not so influential in so far as the injections were concerned as was the number of rainy days.[9]

The diagram shows the considerable capacity of the chestnut tree for absorbing chemical solutions.

The *mean* amount absorbed by a tree per day in a 7-day-long injection, was in April 103 cc.; in May 255 cc.; June 299 cc.; July 201 cc.; August 229 cc.; September 224 cc.; and in October 178 cc.

Comparing the records of the intake of the trees with the weather records, it can be seen that the amount of intake is dependent on the stage of development of the trees, which in turn is dependent on the periodic change of weather during the season. The greater the capacity for transpiration, the larger the initial amount of intake. The irregularities of the curves are due to transient changes of weather modified in turn by the changing capacity for transpiration.

From these records of 1913, it appears that the most favorable month for injection of chestnut trees, so far as rate of intake is concerned, was June; after this month came, in rank, July, May, August, September, October, and April.

Summary

A compilation of the records of injections made in 156 Paragon chestnut trees during the growing season of 1913 shows that the trees possessed a considerable capacity for absorbing solutions of substances.

June was the best month for injection in so far as rate of intake was concerned, then came July, May, August, September, October, and April. The rate of intake varied more in April, May, and June than in the summer and autumn months.

Solutions of organic compounds went into the trees more readily than solutions of inorganic compounds, the "true solutions" more readily than the colloidal.

Injected solutions, with a very few exceptions, were absorbed more readily than injected water.

The more concentrated the solutions of chemicals were, the more readily they were absorbed by the trees.

The effects of the injections here described upon the trees and upon *Endothia parasitica* will be discussed in a later paper.

Bureau of Plant Industry,
U. S. Department of Agriculture,
Washington, D. C.

[8] The rainfall during the period of injection work was 24.7 inches.

[9] For example, in July only 3.1 inches of rain fell, but there were 13 rainy days, the amount of solution absorbed per tree per day dropped during July to 201 cc. In May 4.5 inches of rain fell, with 7 rainy days; the absorption per tree per day was 255 cc. In August 5.8 inches of rain fell with 6 rainy days, and the absorption per tree was 229 cc. per day.

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THE OCCURRENCE AND INHERITANCE OF SEX INTERGRADATION IN PLANTS

Cecil Yampolsky

In a previous paper (1919) I have called attention to sex intergradation in *Mercurialis annua* in both male and female cultures. It is my purpose here to discuss the general question of sex intergrades as they occur in the flowering plants. This discussion is based in a large measure upon the results reported in the paper mentioned above.

There can be no question from the data at hand that sex in Mercurialis is a fluctuating rather than a fixed character expressing itself in a wide range of sex intergrades, including as the extremes some pure male and some pure female plants and midway between the extremes highly fertile monoecious forms. The sex intergrades here are all highly and equally fertile, and no suspicion of abnormality or of pathological conditions can attach to them. That there is a tendency to pure dioecism seems highly probable, but the transition from hermaphroditism is still represented by all possible gradations, showing most convincingly that theories of sex determination based on the segregation of fixed unit factors can have no significance for such types.

Sex Intergrades

Goldschmidt (1916*a*) reports in a preliminary paper upon the sex ratios in crosses between the European and Japanese races of the gypsy moth, *Lymantria dispar*. He obtains various gradations in the sexual condition unlike the well known gynandromorphs. His individuals do not, as in the case of the gynandromorphs, show a sectorial arrangement of the characters of the two sexes, but they do show different gradations between the extremes of femaleness and maleness. His females show all the transition stages, such as feathered antennae, male wing pigmentation, the transition of ovaries into testes, and the loss of the power to lay eggs. His males show tendencies towards femaleness in a similar manner. For these individuals he proposes the term *intersexes*. He finds that as his sex intergrades approach the middle line between maleness and femaleness, they become more and more sterile, that is he obtains no fertile hermaphrodites such as occur for example in *Mercurialis annua*. In fact, although his forms show morphological intersexualism, they are functionally sexless in many instances. To be sure, he secures his intergrades by using as parents forms which in themselves possess functional sexual intergradation. As noted in my previous paper, there is a tendency in plant forms that exhibit gradations in sex (judged by the proportion of male, female, and hermaphro-

ditic flowers) to show a definite influence of that condition upon the sex ratios in the offspring.

Banta (1916) reports the appearance of intersexes in a phyllopod, *Limocephalus vetulus.* The females reproduce parthenogenetically. "In one of the strains there appeared a large percentage of males together with normal females and a large number of sex intergrades—males with one or more female secondary sex characters, females with one to several male characters, and some hermaphrodites with various combinations of male and female secondary sex characters." Eight secondary sex characters distinguish the male from the female. The highly male-like female intergrades are relatively infertile. The more the female takes on the male characters the less likely she is to be fertile. Some individuals with several secondary male characters prove to be very fertile. "In general in addition to being more prolific one may say that female intergrades with few or less distinctly male characters produce a smaller percentage of males and sex intergrades than those having a larger number of more definitely male characters." Males that show one or more female secondary sex characters nearly always have an incompletely developed reproductive system. By propagating from female intergrades, Banta was able to secure the production of mixed broods, males, females, and sex intergrades. The stock derived from these females consists of 40 percent normal males, 8 percent normal females, and the rest intergrades with almost any combination of male and female secondary and primary sex characters. Some of his sex intergrades (female) may parthenogenetically produce normal females and occasionally normal males.

In a later paper, Banta (1918) reports on sex intergrades in *Daphnia longispina.* In this form the male differs from the female in eight secondary sex characters. In Daphnia there are fewer male than female intergrades. The offspring of the more highly male female intergrades tend to be like the mother. A female from a sex intergrade will produce offspring very much like herself with few male secondary sex characters. The more male the female intergrade, the more sterile she is likely to be.

Banta makes the following suggestive remark: "From such clear cases of sex intermediates one wonders if maleness and femaleness are really mutually exclusive in those Cladocera individuals which morphologically show no unlike sex characters. Even in 'normal' strains one is certainly justified in thinking that maleness and femaleness are not complete and mutually exclusive states but that in these apparently normal sex forms, too, sex is also relative—differing from so-called sex intergrades not in kind but merely in degree, not qualitatively but quantitatively."

Plants show most clearly that maleness and femaleness in the same individual do not tend to neutralize each other and to produce sterility. The appearance of intersexes or sex intergrades in the plant kingdom, while not designated by these terms, has been described for very many forms in the

phanerogamic floras. A definite terminology is used in botanical literature to cover this phenomenon. The terms *hermaphroditic, dioecious, monoecious, andromonoecious, gynomonoecious, trimonoecious, gynodioecious, trioecious, androdioecious,* etc., are used.

A hermaphroditic form is one in which both pistils and stamens are borne in the same flower (perfect flower), and in which all the flowers on a plant show the same arrangement of parts. Example: Lilium.

A dioecious form is one in which the sexes are completely separated so that one plant bears male flowers only and the other plant female flowers only. Example: *Elodea canadensis.*

A monoecious form is one in which the pistillate and staminate inflorescences are borne separately on the same plant. Example: Begonia.

An andromonoecious form is one that bears perfect flowers and male flowers on the same plant. Example: many Umbelliferae (Lotsy, 1911).

A gynomonoecious form is one that bears perfect flowers and female flowers on the same plant. Example: Atriplex and many Compositae (Lotsy, 1911).

A trimonoecious form is one that has three distinct types of flowers, male, female, and hermaphroditic. Example: *Acer campestre* (Lotsy, 1911).

A gynodioecious form is one that has both female and hermaphroditic individuals. Example: *Plantago lanceolata.*

An androdioecious form is one that has both male and hermaphroditic individuals. Example: *Caltha palustris* (Lotsy, 1911).

Other combinations may occur. Staminate and pistillate inflorescences may appear on one individual and hermaphroditic on another. Example: Callitriche (Lotsy, 1911). Male and female inflorescences may appear on one individual while another individual may be male. Example: Ceretopus (Lotsy, 1911). Male and female inflorescences may appear on one individual while another may have only female flowers. Example: Morus (Lotsy, 1911). Hermaphroditic and male flowers may appear on one individual while another is female only. Example: Gleditsia (Lotsy, 1911).

A trioecious form is one in which three distinct sex types occur, male, female, and hermaphroditic. Example: hemp.

Correns (1913) offers the following diagram to show these conditions.

Hermaphroditic
☿

Andromonoecious ☿ ♂ | Trimonoecious ♂ ♀ ☿ | Gynomonoecious ☿ ♀

(Male) ♂ Androecious | (Female) ♀ Gynoecious

He proposes to call these forms *mixed in sex*. The old term is *polygamous*. I have used the term *intersexes* or *sex intergrades*.

These groups indicate in every case the distribution of functional sex elements. We find, however, just as Goldschmidt found in his intersexes, so here, that every possible degree of functional and structural perfection and degeneration exists and has been long known, though botanists have been inclined to take them as matters of course until the development of the recent efforts to make sex determination a matter of absolutely alternative inheritance and to represent sex characters by fixed and invariable factors in the germ plasm which are segregated in the reduction division. We can classify the grades of development of female sex organs as follows. In each case functional stamens are present in the same flower or elsewhere on the same plant.

I. Functional Variation.
- *a.* Ovaries fertile to pollen from another genus: *Zea Mays* L. × *Euchlaena Mexicana* Schrad.
- *b.* Ovaries fertile to pollen from one or more different species: *Antirrhinum molle* × *A. majus*.
- *c.* Ovaries both cross- and self-fertile: *Zea Mays*.
- *d.* Ovaries cross-fertile and self-fertile: *Pyrus malus*.
- *e.* Ovaries normal in appearance but both cross- and self-sterile: Eschscholtzia.

II. Structural Variation.
- *a.* Ovaries normal in size, all ovules functional: *Lilium canadense*.
- *b.* Ovaries normal in size, ovules more or less aborted: Phaseolus.
- *c.* Ovaries normal in size, all ovules aborted: *Trifolium pratense*, Syringa hybrids.
- *d.* Ovaries visibly degenerate: *Fraxinus excelsior*.
- *e.* Ovaries mere rudiments: *Fraxinus excelsior*.
- *f.* Ovaries becoming stamen-like: *Salix caprea*.
- *g.* Ovaries not present, replaced by petals: Matthiola.

In the same way we can classify the grades of development of the male sex organs. In each case functional ovaries are present in the same flower or elsewhere on the same plant.

I. Functional Variation.
- *a.* Pollen capable of fertilizing ovaries of another genus: *Zea Mays* × *Euchlaena Mexicana* Schrad.
- *b.* Pollen capable of fertilizing ovaries of another species: *Antirrhinum molle* × *A. majus*.
- *c.* Pollen both cross- and self-fertile: *Zea Mays*.
- *d.* Pollen cross-fertile and self-sterile: *Pyrus malus*.
- *e.* Pollen normal in appearance but both cross- and self-sterile: Eschscholtzia.

II. Structural Variation.
- *a.* Anthers normal in size, all grains functional: *Lilium canadense*.
- *b.* Anthers normal in size, not all pollen grains functional: Phaseolus.
- *c.* Anthers normal in size, some pollen grains aborted: Oenothera hybrids.
- *d.* Anthers visibly degenerate: *Thymus vulgaris*.
- *e.* Anthers mere rudiments: *Echium vulgare*.
- *f.* Anthers becoming pistil-like: *Salix caprea*.
- *g.* Anthers not present, replaced by petals: Matthiola.

Further, as the classification shows, a great many plants exhibit gradations between maleness and femaleness and hermaphroditism because of the more or less nearly complete degeneration or modification of parts. A female may arise from a hermaphrodite through the more or less complete suppression or degeneration of stamens. Likewise a monoecious individual may become female by the degeneration or the entire suppression of the stamens. A male may arise from either one of such forms by the disappearance or more or less nearly complete degeneration of the carpels. When in a group of hermaphrodites the stamens of some of the plants are suppressed or degenerated we have a condition of gynodioecism, if the carpels are suppressed or degenerate a condition of androdioecism. When only parts of the plants exhibit the phenomena described we find a multiplicity of combinations. A gynomonoecious individual may arise from a hermaphrodite in which female flowers appear through the suppression or degeneration of the stamens. An andromonoecious individual may arise from a hermaphrodite through the suppression or degeneration of some of the carpels. Gynomonoecism and andromonoecism, just as well as gynodioecism and androdioecism, may appear in various gradations; in the former instance by virtue of the suppression or degeneration of larger or smaller numbers of parts of the plant, and in the latter case because of changes that may occur in larger or smaller numbers of individuals in a group of plants.

The appearance of sporadic female or male flowers on a hermaphroditic plant may bring about a condition of gynomonoecism or andromonoecism without necessitating the degeneration of parts. Thus, also, among dioecious forms the appearance of male flowers on the pistillate plants or of female flowers on the staminate plants gives all possible sex combinations found in plants.

There is still another form in which a so-called polygamous condition may exist. In these cases either the female or male elements, although morphologically perfect, are physiologically, either one or both, functionless. We find also gradations in the degree of sterility of stamens or ovaries of parts of the plant, of the whole plant, or of varying numbers of individuals in a group of plants.

Earlier investigators who have observed pistillody of the stamens, staminody of the pistils, the appearance of male flowers on female plants, etc., considered them monstrosities and grouped them as such. Moquin-Tandon (1841) and Masters (1869) include all such phenomena under teratology. The many cases of this sort reported for plants would suggest that this treatment is by no means adequate.

Wehrli (1892), who reports on a case of the complete transformation of a male catkin of *Coryllus avellana* L., has brought together all the available literature from 1741 to 1892. He lists over 80 distinct species, monoecious, dioecious, and hermaphroditic, in which such modifications of floral parts have occurred. The phenomena he observed include: the appearance of

male flowers on female plants, of pistils and stamens in the same catkins, appearance of perfect flowers on male plants, abortion of stamens, pistillody of stamens, staminody of pistils, yearly changes in flowers on a tree (nutmeg), sectorial arrangement of male and female inflorescences in *Pinus alba*, and many more. Wehrli's references and *résumé* are so complete that they have not been repeated here. However, a number of typical examples will be listed from the literature since Wehrli's paper.

Haring (1894) gives an elaborate series of drawings showing various gradations in the transition stages of stamens into pistils and of pistils into stamens in *Salix caprea* L. and *S. cinerea* L. He observes that his work shows the tendency in willows to the greatest plasticity in the structure, form, and sex of the floral organs, including the growing together or the separation of parts, the replacement of one sex organ by that of the opposite sex, and the transition of one sex into the other. The author goes on to say that the phenomena that he has described show the morphological equivalence of the organs of both sexes, in the position of the sex organs no matter whether male or female, in the replacement of the organs of one sex by those of the other, and in the transition of one sex into the other.

In the plant kingdom not only is there a transformation of one sex organ into the opposite but the transmuted organs are quite regularly functional, though sterility of the intergrade organs is not uncommon. Intergradation of sex in plants, if measured in percentages, may be from a fraction of one percent to one hundred percent, in the former case by the pistillody of a single stamen or staminody of a single pistil on a whole plant and in the latter case through the complete alteration of a male plant into a female. Sex intergradation as evidenced by the appearance of one or more parts of the opposite sex on a given plant does not seem to affect the fertility of the plant.

We may note in more detail some of the most carefully studied forms which show these sex intergrades. *Satureja hortensis* is described by Correns (1904) as occurring in three forms: (1) plants with female flowers; (2) plants with hermaphroditic flowers, hermaphroditic flowers with shriveled anthers, and female flowers; (3) plants with hermaphroditic flowers and shriveled anthers, and female flowers. These shriveled anthers indicate a tendency to abortion or infertility of the organs of one or the other sex, paralleling the conditions in Goldschmidt's sex intergrades.

Dimorphotheca pluvialis is trimorphic (Correns, 1913). In an earlier paper (1906) he describes the ray flowers as female, the outer disc flowers as hermaphroditic, and the innermost as male.

Correns (1904) finds five forms of *Silene inflata*: males, females, hermaphrodites, gynomonoecious, and gynodioecious individuals.

Wittrock (1886) describes five different kinds of inflorescences in *Acer platanoides*: (1) individuals exclusively female, (2) individuals whose first flowers are female and their later flowers male, (3) individuals whose first

flowers are male and their later flowers in part male and in part female, (4) individuals whose first flowers are male and their later flowers female, (5) individuals exclusively male.

Schulz (1892), on the basis of eleven years' observation of the ash (Fraxinus), recognizes ten distinct forms:

(1) Individuals which bear only male flowers.

(2) Individuals which bear only hermaphroditic flowers.

(3) Individuals which bear only female flowers.

(4) Individuals which bear only male flowers one year and the next year show branches of both male and female flowers.

(5) Males which have certain branches either female, hermaphroditic, or with both kinds of inflorescences.

(6) Individuals which one year bear only female flowers, and the next year have branches with more or less hermaphroditic and female flowers.

(7) Individuals bearing equal numbers of female and hermaphroditic flowers on the same or different branches.

(8) Individuals which bear one year only hermaphroditic flowers and almost always associated with them female flowers, later producing male flowers.

(9) Female or hermaphroditic individuals with male branches.

(10) Individuals with about equal numbers of male, hermaphroditic, and female flowers.

Correns (1908) says that there are at least thirty intergrading categories recognizable in *Plantago lanceolata*. In his classification of forms for experimental purposes he recognizes the following classes: (1) hermaphrodites, (2) predominantly hermaphrodites, (3) hermaphrodites and females, (4) predominantly females, and (5) females.

I have given only a few examples of the very many that are listed in the plant kingdom, but the forms cited are sufficient to show the wide range of intersexuality that exists among plants. These cases of intergrades in functional and structural development of the sex organs, taken in connection with the classes based on the distribution of the sex organs by plants as individuals as tabulated above, present an almost bewildering completeness as a picture of the theoretically possible gradations in sex characters both of the gametes and of the organisms which produce them. And it is to be remembered that for the most part these are not exceptional or chance cases. They represent the common and obvious facts as to sex in the flowering plants. No theory of sex based on the assumption of the alternative inheritance of fixed sex factors which are segregated at the time of the reduction divisions can do justice to the conditions presented in the higher plants.

I have brought together data as to the distribution of sex forms in the various orders of seed plants. For this purpose I have followed Engler and Gilg's "Syllabus der Pflanzenfamilien." Practically every order has fami-

lies which contain forms that show more than one kind of distribution of the sex elements. Thus in the monocotyledons ten of the eleven orders have hermaphroditic, monoecious, dioecious, and polygamous individuals. There are twenty-two families represented in the ten orders. In the dicotyledons thirty-one of the forty orders have representatives of two or more of the various distributions of the sex elements. There are ninety families that exhibit this tendency. At the end of the paper are listed the families and the sex forms found in each. Their distribution is further shown by means of a table.

Change of Sex Apparently as a Result of Environmental Influences

Changes of sex from year to year and apparently as a result of environmental influences are inextricably interrelated with the fluctuations of maleness and femaleness in sex intergrades and must hence be briefly considered here.

Gallardo (1901) reports on the work of Spegazzini, who by transplanting wild female plants of Dioscorea, Clematis, and Trianosperma found that the following year fruit was set. Examination showed that these plants bore either male or hermaphroditic flowers besides the female flowers. The following year, however, they became female again. Male plants, transplanted, showed no change of sex.

De Vries (1903) figures the appearance of seeds on a male branch of *Mercurialis annua*. Strasburger (1910) cut back 200 male plants to ascertain whether severe pruning would have any effect upon them. Only one male plant that had been cut back produced a single female flower. One of his plants, no. 16, started as a pure female. It began, however, gradually to develop male flowers with functional pollen. It became more and more male, producing the characteristic odor of the male plants. He collected 55 seeds from this plant but only 5 germinated, 2 males and 3 females being produced. This behavior of Strasburger's plant, with reference to the production of a mixed progeny, might perhaps be explained on the basis that the seeds set when the plant was predominantly female produced female offspring, while the seed produced when the plant was predominantly male produced males. The 55 seeds may even have represented the three conditions, male, female, and hermaphroditic.

Higgins (1916) reports a case in which a male plant of *Carica papaya* was cut down, leaving only a stump. This stump sent out branches which bore abundant fruit.

Pritchard (1916) found that by mutilating male and female plants of hemp the appearance of organs of the opposite sex could be induced. The author calls attention to the presence of monoecious individuals as a normal occurrence, often constituting as high as eight percent of the dioecious cultures.

Davey and Gibson (1917) have found in Myrica, which is described as

dioecious, gradations in sex like those described for other forms. They find a small proportion of monoecious plants which represent all gradations between the normal pistillate and staminate types. They also describe bushes and shoots whose sex may vary from year to year. Fourteen cases found to be entirely pistillate in 1913 and 1914 produced staminate catkins in 1915. One plant produced almost entirely staminate catkins. Certain trees and branches which produced abundant fruit in 1913 developed mixed shoots in 1914 and in 1915 became almost staminate.

The classic case of alteration of sex in plants is that of *Lychnis dioica* when attacked by the anther smut fungus, *Ustilago violacea*. Strasburger (1900) points out that both male and female plants are attacked by the smut. In the anthers of the male the parasite causes a characteristic purplish color, the interior of the anther being filled with smut spores. In the female the fungus causes a more profound change. The plant is stimulated to produce anthers with the characteristic sporogenous tissue which tissue is later destroyed by the fungus so that the anther is ultimately filled with fungus spores.

Although the list which I have brought together is by no means complete, it is, however, sufficiently representative of the changes in sex that have been reported in the literature. Sex intergrades, it will be noted, may occur in various degrees, from the transition of one sex organ into that of the opposite sex to a complete change of sex of the entire plant.

Secondary Sex Characters

It is to be noted that intersexualism in animals is measured by the degree of modification of one or the other of the secondary sex characters, by the appearance of secondary sex characters of one sex in individuals of the opposite sex, as well as by the degeneration of ovary or testis or the transition of an ovary into a testis or of a testis into an ovary. In animals sex dimorphism is the characteristic thing, and one is familiar with such differences in sex as size, voice, stature, plumage, and the like.

Sex dimorphism in flowering plants, where the sexes are separate, is not very striking; secondary sex characters have been contrasted but little in such forms. Darwin (1889, page 11) cites the case of the Resteaceae of Australia and the Cape of Good Hope, forms which show extreme sex dimorphism. It is reported that often it is impossible to match the male with the female of the same species. Shull (1914) reports for *Lychnis dioica* L. a sex-limited character in the form of narrowness of leaf in the male of *Lychnis dioica angustifolia*. Cook (1914) reports on a case of sex inequality in hemp, where the male plants are smaller and shorter than the females. These male plants die much sooner than the females.

The female inflorescences of Mercurialis are borne in clusters in the axils of the leaves, while the male inflorescences are borne in interrupted spikes which surpass the leaves. This characteristic appearance of the

inflorescences of the two sexes may be considered as the secondary sex character of the two sexes, in the sense that the manner in which the inflorescences are borne is characteristic for each sex. No doubt a closer examination of other dioecious forms will show differences in male and female pedicels, petals, and sepals, either by their presence or by their absence.

It is interesting to note that in sex intergradation in *Mercurialis annua* there is no transition of a secondary sex character of one sex into that of the other. Those females which tended towards maleness by producing many male flowers and many seeds did not take on the general growth characteristics of the male. The same holds true for the males that tended towards femaleness—they too still maintained their characteristic form of growth.

The Doctrine of Varying Potencies in Germ Cells

Alternative inheritance of sex is the extreme of a series of intergraded variations. Hermaphrodites (with perfect flowers) and monoecious forms become dioecious not by the sudden development of heterozygosis in one sex and the separation of sex factors in the reduction division, but by the gradual development of sex purity (dioecism) through a long series of intergraded sex variants. The connecting links can all be found in the polygamous (mixed) species. If dioecism has arisen in this way, it is hardly likely that there is anything of the nature of fixed, invariable sex factors in the germ plasm. It is a matter of fluctuating tendencies. Male tends to produce male, female to produce female; sometimes one tendency is stronger, sometimes the other.

Strasburger (1910) attacks Correns' view that one sex is heterozygous for a sex determiner on phylogenetic grounds. The evolutionary trend has been to make the egg and sperm different. Phylogeny points to the egg's being female-producing and the male gamete's being male-producing. It is certainly an awkward assumption that one half the male gametes, for example, must carry female determiners. In an earlier work Strasburger (1909*a*) concluded that the egg of the dioecious phanerogam tends to produce females only, while in the production of the microspores of a tetrad by division of the pollen mother cell two of the spores will have a stronger male tendency than the other two. Those with the stronger male tendency (which is transmitted to their descendants, the male gametes) will dominate over the female tendency of the egg and thus males will be produced, while the weaker male tendency of the other two will be dominated by the stronger female tendency of the egg and females will result. Noll (1907), from his studies of dioecious plants, was led to this view that there are pollen grains of two strengths as regards the male-producing tendency.

While Strasburger's view explains the behavior of his selfed females and of his selfed males, and the sex of the progeny resulting from the fertilization of a female by a male, there is one difficulty that he overlooked. Assuming that the eggs are all of one kind, then the eggs produced on the

male plants must all dominate over the weaker male gametes. Such however, is not the case. In the female that produces sporadic male flowers there is no reason, on Strasburger's assumption, why the male gametes should not be of two kinds. Selfed females produce only female offspring. That means that the male-producing tendencies transmitted by all the pollen grains of the tetrad are dominated by the female-producing tendency of the egg. On Strasburger's assumption there must now be at least three strengths of pollen grains, if not four: two kinds produced by the male, one of which is subordinate in its sex-determining tendency to the egg, and two kinds (on *a priori* grounds) produced by the sporadic male flowers on the female. Then, too, there are two kinds of eggs instead of one kind: the egg of the female plant which dominates over the weaker male-producing tendency of the pollen grains, and the egg produced upon the male plants, which is dominated by the male-producing tendencies of both kinds of pollen grains, and is thus weaker than the eggs borne on the female plant.

We reach here a conception, which the thus-far meager data on inheritance in dioecious and polygamo-dioecious forms seem to bear out, namely, that *there may be graded potencies in both the gametes, the egg as well as the male gamete, of such forms.* The work of Correns is especially significant. In his work on Satureja, Silene, and Plantago he brings out clearly that the more pronounced the sex of the individual the more marked will be its influence on the sex of its offspring. The normal appearance of sex intergrades (there are at least thirty degrees in *Plantago lanceolata* between pure female and hermaphrodite) is evidence in that direction. The behavior of the females of Mercurialis in my cultures is interesting in this respect. The original mother plant produced 66 seeds and 50 offspring. The offspring in turn produced seeds varying in number from 1 to 238. The original mother plant produced eggs of varying potencies as evidenced by the variation in male flower and seed production of the offspring. It is quite natural that the eggs should have varied in their ability to transmit the seed-producing qualities of the mother as in other qualities. Although the offspring tended to be like the mother in the sense that they were pure females or predominantly females, they varied in their ability to produce male flowers and hence seeds. The fertilized egg that produced a female that during its life history produced no male flowers or seeds is different, whether it be qualitatively or quantitatively, from the fertilized egg that produced a plant that produced many male flowers and seeds. One can conceive gradations in the power to produce male flowers and seeds, beginning with eggs with zero potentiality and running thence all the way to those with the potentiality of plant no. VII (Yampolsky, 1919), which produced 32 male flowers and 236 seeds.

The male cultures of *Mercurialis annua*, while they do not show the tendency toward intergradations as often as do the females, nevertheless bring out very clearly gradations in sex potency.

On the assumption that gametes vary or are graded in strength, the one-to-one ratio may be explained in dioecious forms, especially in dealing with mass populations. As has already been pointed out (*l.c.*), it is only when large numbers are considered that the one-to-one ratio appears. To be sure the law of chance comes into effect in such an explanation of sex ratios. The explanation of the one-to-one ratio may very well lie in the assumption that the gametes of the female have as much chance to dominate over the male gametes as the male gametes have to dominate over the female. That the gametes of one sex may in cases completely or almost completely dominate over those of the other sex is brought out in aberrant sex ratios. This advantage may, when large numbers of individuals are considered, be offset by a parallel condition resulting in the dominance of gametes of the other sex (Doncaster, 1913, 1916; Montgomery, 1908).

In Mercurialis, though the species is prevailingly dioecious, it is obvious that we must assume that the potentialities for the development of both sexes are present in practically all the individuals of the species. There is nowhere evidence that sex is determined in this plant by the presence or absence of a sex-determining factor. Those individuals which remain purely male or purely female throughout are not to be conceived as very different from those which produce a few flowers of the opposite sex. There is no evidence for the localization of the sex difference either in a special part of the plant or in a special part of the cell. The appearance of the sporadic flowers of the other sex may occur anywhere on the plant and at any stage of its development. Their occurrence is comparable to that of bud variation, and like the latter they show that the organism may contain latent potentialities as well as visibly expressed characters. Nor does the production of a few flowers of the other sex alter essentially the sex character of the plant as a whole. It is still prevailingly male or female and transmits its sex as such. It is highly probable that as a rule at least the pollen from sporadic male flowers on a female plant pollinates the nearest female flowers on the same branch. The seeds so produced, however, grow into female plants like the branch which bore them. It is sometimes questioned whether a plant with its potentialities of unlimited growth and with its succesive crops of reproductive organs is an individual in the sense that an animal is, with its more limited growth and definitely localized reproductive and other organs. The behavior of these prevailingly dioecious Mercurialis plants with reference to sex transmission certainly shows that they are unit individuals male or female in a very strict sense. But it is just as clear that, as noted above, the dioecious condition is only an extreme, a climax condition in the evolution of sex differentiation. As the data at the end of this paper show, the transition from the hermaphroditic and monoecious to the polygamo-dioecious and dioecious condition is going on at numerous and widely distributed points in the orders and families of seed plants.

Distribution of Sex Forms According to Engler and Gilg

Monocotyledons
- Order Pandanales
  - Pandanaceae: monoecious, dioecious, polygamo-dioecious
- Order Helobiae
  - Potamogetonaceae: monoecious, dioecious, hermaphroditic
  - Najadaceae: monoecious, dioecious, hermaphroditic
  - Scheuchzeriaceae: monoecious, dioecious, hermaphroditic
  - Alismataceae: monoecious, dioecious, hermaphroditic
  - Hydrocharitaceae: monoecious, dioecious, hermaphroditic
- Order Triuridales
  - Triuridaceae: monoecious, dioecious, hermaphroditic
- Order Glumiflorae
  - Gramineae: monoecious, dioecious, hermaphroditic
  - Cyperaceae: monoecious, dioecious, hermaphroditic
- Order Principes
  - Palmae: monoecious, dioecious, hermaphroditic
- Order Spathiflorae
  - Araceae: Monoecious, dioecious, hermaphroditic
- Order Farinosae
  - Flagellariaceae: monoecious, hermaphroditic
  - Restionaceae: dioecious, hermaphroditic
  - Centrolepidaceae: monoecious, hermaphroditic
  - Eriocaulaceae: monoecious, dioecious, polygamous
  - Commelinaceae: monoecious, hermaphroditic
- Order Liliiflorae
  - Liliaceae: mostly hermaphroditic, dioecious (Smilax, Britton)
  - Dioscoreaceae: monoecious, dioecious, hermaphroditic
- Order Scitamineae
  - Musaceae: monoecious, hermaphroditic
  - Zingiberaceae: monoecious, hermaphroditic
  - Marantaceae: monoecious, hermaphroditic
- Order Microspermae
  - Orchidaceae: mostly hermaphroditic
    - (Cataseteae): hermaphroditic, monoecious

Dicotyledons
- Order Piperales
  - Piperaceae: monoecious, hermaphroditic
  - Chloranthaceae: monoecious, hermaphroditic
- Order Salicales
  - Salicaceae: monoecious, dioecious
- Order Myricales
  - Myricaceae: monoecious, dioecious
- Order Balanopsidales
  - Balanopsidaceae: dioecious
- Order Leitneriales
  - Leitneriaceae: dioecious
- Order Batidales
  - Batidaceae: dioecious
- Order Julianiales
  - Julianiaceae: dioecious

Order Fagales
- Betulaceae: monoecious, rarely dioecious
- Fagaceae: monoecious, rarely hermaphroditic

Order Urticales
- Ulmaceae: monoecious, dioecious, polygamous, hermaphroditic
- Moraceae: monoecious, dioecious
- Urticaceae: monoecious, dioecious, polygamous, hermaphroditic

Order Proteales
- Proteaceae: monoecious, hermaphroditic

Order Santalales
- Santalaceae: monoecious, dioecious, hermaphroditic
- Loranthaceae: monoecious, dioecious, hermaphroditic

Order Aristolochiales
- Rafflesiaceae: monoecious, hermaphroditic

Order Polygonales
- Polygonaceae: monoecious, dioecious, polygamous, hermaphroditic

Order Centrospermae
- Chenopodiaceae: monoecious, dioecious, hermaphroditic
- Amarantaceae: rarely monecious, dioecious, polygamous, hermaphroditic
- Nyctaginaceae: monoecious, hermaphroditic
- Phytolaccaceae: monoecious, polygamous, hermaphroditic
- Caryophyllaceae: monoecious, dioecious

Order Ranales
- Ceratophyllaceae: monoecious, dioecious
- Trochodendraceae: monoecious, hermaphroditic
- Cercidiphyllaceae: dioecious
- Ranunculaceae: dioecious, hermaphroditic
- Lardizabalaceae: monoecious, hermaphroditic
- Menispermaceae: dioecious
- Magnoliaceae: monoecious, hermaphroditic
- Monimiaceae: monoecious, hermaphroditic
- Lauraceae: monoecious, dioecious, polygamous, hermaphroditic
- Hernandiaceae: monoecious, hermaphroditic

Order Rosales
- Hydrostachyaceae: dioecious
- Saxifragaceae: polygamo-dioecious, hermaphroditic
- Hamamelidaceae: monoecious, polygamous, hermaphroditic
- Rosaceae: polygamo-dioecious, hermaphroditic
- Connaraceae: monoecious, hermaphroditic
- Leguminosae: monoecious, dioecious, polygamo-dioecious, hermaphroditic

Order Pandales
- Pandaceae: dioecious

Order Geraniales
- Rutaceae: polygamo-dioecious, hermaphroditic
- Simarubaceae: polygamous, dioecious, hermaphroditic
- Burseraceae: dioecious, hermaphroditic
- Dichapetalaceae: dioecious, hermaphroditic
- Euphorbiaceae: monoecious, dioecious
- Callitrichaceae: monoecious, hermaphroditic

Order Sapindales
- Buxaceae: monoecious, dioecious
- Empetraceae: monoecious, dioecious, polygamous
- Coriariaceae: monoecious, hermaphroditic

Anacardiaceae: polygamo-dioecious, hermaphroditic
Aquifoliaceae: dioecious, polygamo-dioecious
Salvadoraceae: dioecious, hermaphroditic
Icacinaceae: monoecious, hermaphroditic
Aceraceae: dioecious, polygamous
Hippocastanaceae: polygamous
Sapindaceae: polygamo-dioecious
Sabiaceae: polygamo-dioecious, hermaphroditic

Order Rhamnales
Rhamnaceae: polygamous, hermaphroditic
Vitaceae: polygamo-dioecious, hermaphroditic

Order Malvales
Sterculariaceae: monoecious, hermaphroditic

Order Parietales
Dilleniaceae: monoecious, hermaphroditic
Guttiferae: monoecious, hermaphroditic
Calophylloideae: monoecious, hermaphroditic
Flacourtiaceae: monoecious, dioecious, hermaphroditic
Stachyuraceae: polygamous, hermaphroditic
Passifloraceae: monoecious, hermaphroditic
Datiscaceae: monoecious, dioecious, hermaphroditic

Order Myrtiflorae
Elaeagnaceae: monoecious, dioecious, polygamous, hermaphroditic
Sonneratiaceae: monoecious, hermaphroditic
Nyssaceae: monoecious, hermaphroditic
Combretaceae: monoecious, hermaphroditic
Halorrhagaceae: monoecious, dioecious, hermaphroditic
Cynomoriaceae: monoecious, hermaphroditic

Order Umbelliflorae
Araliaceae: monoecious, polygamous, hermaphroditic
Umbelliferae: monoecious, polygamous, hermaphroditic
Cornaceae: monoecious, hermaphroditic

Order Primulales
Theophrastaceae: monoecious, hermaphroditic
Mrysinaceae: monoecious, hermaphroditic

Order Ebenales
Ebenaceae: dioecious, polygamous, hermaphroditic
Styracaceae: hermaphroditic, rarely polygamo-dioecious (Britton)

Order Contortae
Oleaceae: monoecious, dioecious, hermaphroditic
Loganiaceae: monoecious, hermaphroditic
Gentianaceae: monoecious, hermaphroditic

Order Plantaginales
Plantaginaceae: monoecious, hermaphroditic

Order Rubiales
Rubiaceae: rarely monoecious, hermaphroditic
Valerianaceae: monoecious, dioecious, polygamo-dioecious, hermaphroditic

Order Cucurbitales
Cucurbitaceae: monoecious, dioecious, hermaphroditic

Order Campanulatae
Stylidiaceae: monoecious, hermaphroditic
Calyceraceae: monoecious, hermaphroditic
Compositae: monoecious, dioecious, polygamous, hermaphroditic

Numbers of Families in Different Orders Showing the Various Types of Sex Arrangement.

Type I. Dioecious.
Type II. Dioecious, monoecious.
Type III. Dioecious, monoecious, hermaphroditic.
Type IV. Dioecious, hermaphroditic.
Type V. Dioecious, polygamous.
Type VI. Dioecious, polygamous, hermaphroditic.
Type VII. Dioecious, polygamous, monoecious.
Type VIII. Dioecious, polygamous, monoecious, hermaphroditic.
Type IX. Polygamous.
Type X. Polygamous, hermaphroditic.
Type XI. Polygamous, hermaphroditic, monoecious.
Type XII. Monoecious, hermaphroditic.

Order	Types											
	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII
Pandanales							1					
Helobiae			5									
Triuridales			1									
Glumiflorae			2									
Principes			1									
Spathiflorae			1									
Farinosae				1			1					3
Liliiflorae			1	1								
Scitamineae												3
Microspermae												1
Piperales												2
Salicales		1										
Myricales		1										
Balanopsidales	1											
Leitneriales	1											
Batidales	1											
Julianiales	1											
Fagales		1										1
Urticales	–	1	–	–	–	–	–	2				
Proteales												1
Santalales	–	–	2									
Aristolochiales												1
Polygonales								1				
Centrospermae	–	1	1				1	1	–	–	1	1
Ranales	2	1	–	1				1				5
Rosales	1							1	–	2	1	1
Pandales	1											
Geraniales	–	1	–	2	–	1				1		1
Sapindales	–	1	–	1	2	–	1		2	2		2
Rhamnales										2		
Malvales												1
Parietales			2							1		4
Myrtiflorae			1					1				4
Umbelliflorae			3									1
Primulales												2
Ebenales						1				1		
Contortae			1									2
Plantaginales												1
Rubiales								1				1
Cucurbitales			1									
Campanulatae								1				2
Total—Families	8	8	22	6	2	2	4	9	2	9	2	40
—Orders	7	8	13	5	1	2	4	8	1	6	2	21

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AN APPARATUS FOR AUTOMATICALLY CHANGING THE TEMPERATURE OF A CHAMBER

George F. Potter

It has been shown that the injury produced in certain plant tissues by freezing is influenced considerably by the rate at which the temperature falls during the freezing process.[1] In order to obtain a uniform and known rate of temperature fall for experiments of this sort, the writer has developed an apparatus in which the *rate of temperature change* is automatically controlled by clockwork. Any desired rise or fall of temperature can be obtained, the conditions desired for a ten-hour period being determined and recorded in advance. By repeating the experiment without altering the adjustments, different lots of tissues may be frozen under duplicate temperature conditions.

A longitudinal section of the freezing chamber, together with end, top, and side views of the controlling mechanism are shown in Plate I. Details of certain portions of the apparatus, as indicated by lettering analogous to that used in Plate I, are shown in both front and side view in Plate II. The freezer proper consists of three cylindrical galvanized iron cans, placed one within the other, riveted together, and packed in a box of sawdust for insulation. The freezing mixture is placed in the space between the two outside cans (*a*, Plate I). The innermost can (*c*, Plate I) is the freezing chamber. The intervening space (*b*, Plate I) is used as an air space to prevent too rapid cooling unless temperatures lower than—10° C. are desired, in which case it is filled with an additional quantity of ice and salt. The two outside chambers (*a* and *b*, Plate I) are fitted with pipes and stopcocks for drawing off the brine. The opening through these must be straight to facilitate removing obstructions.

The freezing chamber is fitted with a tight galvanized iron cover, to the center of which a short section of iron pipe is attached firmly by means of an iron collar. All thermometers, recording and control apparatus are introduced through this opening. A steel T-bar is securely clamped to the iron pipe (section *BB*, Plate II) and extends from slightly above the top of the pipe to within a few inches of the bottom of the chamber. This bar serves as a support for both fan shaft and thermostat, and by means of clamps holds interchangeable racks to which the experimental materials are attached. All three cans are covered by a single galvanized iron cover of circular shape insulated with cork board. The iron pipe from the inner

[1] Chandler, W. H. Killing of plant tissue by low temperature. Mo. Agric. Exp. Sta. Research Bull. **8**: 199–205. 1913.

chamber passes through an opening in the exact center of the insulated cover, which is therefore free to revolve about the pipe. Ice and salt may be placed in any part of the two outer spaces (*a* and *b*, Plate I), through an opening near the outer edge of this cover.

In such a chamber it is always difficult to keep the temperature uniform from top to bottom. To accomplish this as nearly as possible, a false wall or tin cylinder about two inches smaller in diameter than the freezing chamber, is introduced and held in place with wooden blocks (longitudinal section of freezer, Plate I). The cold wall of the chamber outside and the heating coil within cause the air to circulate upward inside the false wall and downward outside it. In addition, the circulation is forced by a fan at the bottom of the chamber. When one is freezing parts of plants that do not obstruct the passage of air, thermometer readings indicate that there is less than 0.1° C. difference in temperature over a vertical distance of eight inches.

The heating coil is wound with about 30 feet of no. 30 "Chromel C" resistance wire, having a total resistance of about 200 ohms. As a rule a large low-resistance lamp, or two 40-watt lamps in parallel, are placed in series with the coil to reduce the amount of heat given off and to act as a pilot light. On a 110-volt A. C. lighting circuit about one half ampere or less of current is used. The lamp can be switched out of the circuit if more heat is needed, as for instance when a large quantity of freezing mixture has just been added. An ordinary "Dim-a-lite" or "Hylo" connection is also placed in the circuit and can be used to reduce the amount of heat given off by the coil, as for instance when the ice is nearly exhausted. These adjustments are frequently convenient, although not necessary for the operation of the machine.

A mercury thermostat (*C*, Plate II) controls the heating coil by means of a telegraphic relay operating on current from two dry cells. When the machine is operated continuously it is necessary to have two batteries of two cells each. One battery may then recuperate while the other is in use. The connection is conveniently alternated by means of a double throw switch. The thermostat consists of a piece of capillary tubing sealed to a bulb containing mercury. An enlargement at the top of the capillary holds the excess mercury at temperatures above the working range. The thermostat used by the writer has a bulb about 1 cm. in diameter by 13 cm. in length and contains sufficient mercury to cause the mercury in the capillary to rise or fall about 2 mm. for each degree Centigrade change of temperature. Electric connection is made between the mercury in the bulb and that in a side arm by means of a platinum wire sealed in the side of the bulb (*C*, Plate II). A platinum wire guided to the exact center of the capillary by a small glass rod makes contact at the top of the mercury column (*CC*, Plate II). The guide is indispensable for accurate results because it makes an appreciable difference in temperature whether contact is

made at the center or at the edge of the convex meniscus. An insulated wire is run from the battery to the side arm. The metal parts of the apparatus acting as a ground wire carry the current to the contact in the capillary. A condenser is used to prevent sparks at the point of contact with the mercury, and to eliminate the arc completely it is necessary to short circuit the terminals of the condenser through a resistance of about 700 ohms. A fifteen-watt Mazda bulb is used for this purpose.

The point at which the platinum wire touches the mercury in the capillary tube controls the temperature at which the heating coil is brought into use, and hence controls the temperature of the chamber. The contact wire is attached to a plunger, which moves vertically in guides fastened to the T-bar above the thermostat (longitudinal section, Plate I, and *B* and *C*, Plate II). The mechanism which actuates this plunger is similar in principle to a recording thermometer (*E*, *F*, and *G*, Plate I). The hands were removed from a "Big Ben" alarm clock and a shaft, bearing a drum three inches in diameter and four inches long, was soldered to the hour-hand shaft. A time-temperature chart is attached to the drum, degrees being marked by equal spaces along the axis of the cylinder, and hours by twelve equal spaces about its circumference. The distance to be laid off for each degree depends on the sensitivity of the thermostat and on the relative lengths of the two levers which will be referred to as the "long arm" and "short arm" of the "revolving shaft." The time-temperature curve is constructed with a flexible lead bar, fastened at each end by a clamp (*e*) sliding in a groove which runs lengthwise of the drum (*F* and *G*, Plate I). Below the drum and at right angles to it there is a revolving shaft (*E*, Plate I). At the clockwork end of this shaft a long arm, somewhat similar to that which carries the recording pen of the thermograph, extends upward and engages the lead bar with a small connecting pin (*E* and *G*, Plate I). At the other end of the shaft, which is pivoted in a bearing at the top of the T-bar leading down into the freezing chamber, there is a short arm which is joined by means of a connecting rod to the plunger carrying the platinum contact wire of the thermostat (*A* and *B*, Plate II). The weight of the connecting rod and plunger acting on the short arm as a lever tends to revolve the shaft and thus keeps the connecting pin of the long arm in contact with the edge of the lead bar. As the drum revolves, a movement of the long arm is permitted in accordance with the curve traced by the lead bar, and a proportionate movement is transmitted to the plunger and contact point of the thermostat through the turning of the shaft and through the resulting movement of the short arm and connecting rod. The short arm is attached to the shaft in such a way that it forms a right angle with the connecting rod when the long arm is at right angles to the axis of the drum. The vertical movement of the plunger is therefore always exactly proportionate to the horizontal distance which the long arm moves along the axis of the drum, although the amount that the shaft revolves for each degree of temperature is greater as the arm approaches either end of the drum.

Regulation of the apparatus is accomplished by means of an adjusting screw at the top of the connecting rod (*A*, Plate II). The temperature within the chamber is read with a thermometer. The drum is revolved until the long arm indicates the same temperature on the chart. The screw is then turned until the platinum point just makes a contact with the mercury in the capillary. In making this adjustment it is necessary to allow for some "lag" in the thermostat if temperatures are changing rapidly in the chamber and if the thermometer used is a sensitive one. As a rule the apparatus is held about fifteen minutes at the adjusting temperature. In operation, the "lag," using the large thermostat mentioned above, did not cause variations in temperature of more than 0.25° C. when the temperature of the chamber was changing at a rate of 16° C. per hour.

The connecting rod is so designed that it may be lifted up and detached from its support on the short arm of the revolving shaft. The base of the clock mechanism is fastened to a slotted support by a screw clamp. By detaching the connecting rod and loosening the clamp, shaft and clock can be removed. The insulated wire leading to the thermostat is then disconnected from its binding post and the covers of the freezing chamber may be removed.

The use of this machine has enabled the writer to perform freezing experiments under conditions controlled more accurately than has been possible even with the closest personal attention when using a hand-controlled freezer. The range and accuracy of regulation of temperature depend almost entirely upon the thermostat. The instrument used by the writer works through a range of 10° C., and the variations between the temperature indicated on the chart and that observed in the chamber usually are not greater than 0.1° C., although sometimes variations of 0.25° C. are observed. A less sensitive thermostat capable of working through a correspondingly greater range of 40° C. showed maximum variations of about 0.5° C. However, if particles of mercury become separated from the top of the column in the capillary tube, the operation of the machine becomes unreliable and usually the temperature of the chamber becomes too high. To avoid this the capillary must be cleaned of any oil or dirt about once a week, or sometimes oftener if foreign materials chance to enter. *It is also necessary to keep the glass guiding rod of the contact point (CC, Plate II) above the surface of the mercury because if immersed it breaks the column,* carrying part of the mercury up above the rod. It is possible that this difficulty could be eliminated by the use of some other type of thermostat, but the writer has not been able to find one which has the same sensitivity, and at the same time the ability to return accurately to the original starting point after going through wide temperature variations. The last mentioned characteristic is absolutely essential for the operation of a machine of this sort. Even with these limitations the machine enables the experimenter to do much more accurately controlled work than would be possible

without it. The writer's record book shows a total of 74 experiments performed in February and March, 1919. Seven of these were of at least 48 hours duration, about half of the remainder 12 hours, and none less than 6 hours. Most of the time one short experiment was carried on during the day, and one of twelve or more hours' duration was run during the night. Out of the total, seven were discarded because the final temperature was more than 0.25° C. from that desired.

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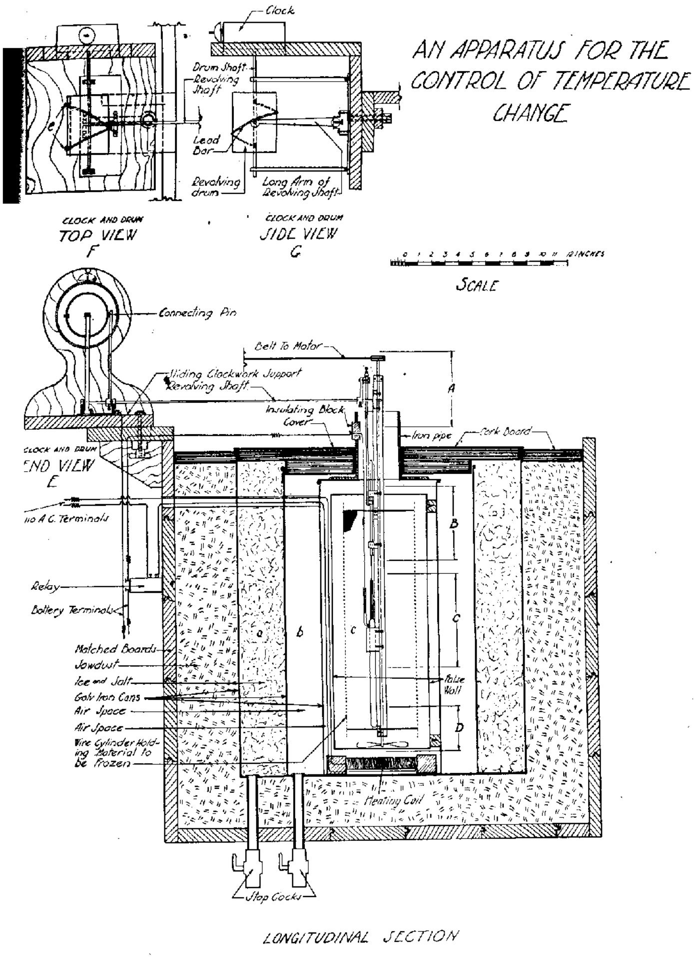
UNIVERSITY OF WISCONSIN



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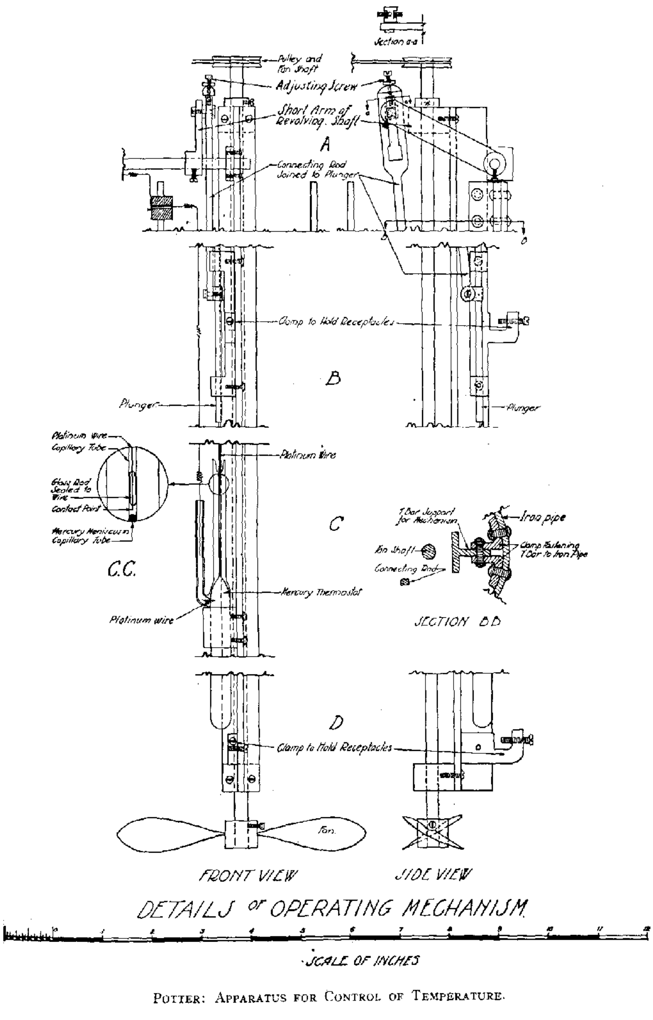

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